

## CLAIMS

What is claimed is :

1. A method of preparation of protein sample solution for analysis, wherein the protein sample solution contains one or more of non-protein agents selected from a group consisting of an anionic detergent, a cationic detergent, a non-ionic detergent, a zwitterionic detergent, a sulfobutane, a lipid, a natural product, a salt, and a common laboratory agent, wherein after the preparation of the protein sample the protein in the sample is quantitatively recovered and is substantially free from the non-protein agents originally present in the sample, comprises the following steps:

(a) treating the protein sample solution with an acidic agent, and one or both agents selected from a group consisting of a salt and a precipitate-forming agent, wherein the treatment of the protein with the precipitate-forming agent follows the treatment of the protein sample solution with the acidic agent;

(b) centrifuge the protein sample solution of the step (a) at least once to form a tight pellet at the bottom of the tube, remove and discard the supernatant and collect a protein pellet;

(c) suspend and mix the protein pellet of the step (b) at least once in at least one medium selected from a group consisting of a mixture of aqueous-organic solvent and an organic solvent;

(d) centrifuge the protein pellet suspension of the step (c) and collect the protein pellet;  
and

(e) suspend the protein pellet of the step (d) in a protein pellet solubilization reagent buffer, wherein the reagent buffer is provided with an acid neutralizing agent in a sufficient amount to substantially neutralize the acid captured in the protein pellet to facilitate a desired protein solubilization.

2. The method of protein precipitation according to Claim 1 wherein the protein sample solution contains an ionic detergent SDS.

3. The method of protein precipitation according to Claim 2, wherein the salt agent is in a amount effective to precipitate the detergent present in the protein solution.
4. The method of Claim 1 wherein the salt is provided in a solution with the acid agent.
5. The method of Claim 1 wherein the precipitate-forming agent is a deoxycholate.
6. The method according to Claim 1 wherein the precipitate-forming agent is soluble and extractable in the organic solvent.
7. The method according to Claim 1 wherein the organic solvent is selected from a group consisting of an acetone and an alcohol.
8. The method of Claim 1 further comprises first suspending the protein pellet of the step (b) in an aqueous medium followed by suspension in the organic solvent.
9. The method of Claim 1 further comprises mixing a polysaccharide solution with the protein pellet of the step (b).
10. The method according to Claim 1 wherein the pellet solubilization reagent buffer is provided with a pH indicator dye.
11. The method of Claim 1 further comprises vigorously agitating and/or grinding the protein pellet suspended in the pellet solubilization reagent buffer in the step (e).
12. The method of Claim 1 further comprises addition of an acid neutralizing agent into the pellet solubilization buffer to shift the pH of the suspension to favor desired protein solubilization.

13. The method of Claim 1 wherein the centrifugation in the step (b) is repeated to remove residual supernatant.

14. The method according to Claim 1 wherein the second centrifugation in the step (b) is performed by placing the tube in the centrifuge in the sample orientation as before.

15. The method of Claim 1 further comprises addition of an acid neutralizing agent to neutralize approximately or greater than 0.25 nM acid per micro-gram protein in the pellet to favor desired protein solubilization.

16. A method of preparation of protein sample solution for analysis, wherein the protein sample solution contains one or more of non-protein agents selected from a group consisting of an anionic detergent, a cationic detergents, a non-ionic detergent, a zwitterionic detergent, a sulfobutane, a lipid, a natural product, a salt, and a common laboratory agent, wherein after the preparation of the protein sample the protein in the sample is quantitatively recovered and is substantially free from the non-protein agents originally present in the sample, comprises the following steps:

(a) treating the protein sample solution with an acidic agent, and one or both agents selected from a group consisting of a salt and a precipitate-forming agent, wherein the treatment of the protein with the precipitate-forming agent follows the treatment of the protein sample solution with the acidic agent;

(b) centrifuge the protein sample solution of the step (a) to form a tight pellet at the bottom of the tube, remove and discard the supernatant and repeat the centrifugation to remove the residual supernatant with a tipped device and collect a protein pellet;

(c) suspend and mix the protein pellet of the step (b) at least once in at least one medium selected from a group consisting of a mixture of aqueous-organic solvent and an organic solvent;

(d) centrifuge the protein pellet suspension of step (c) and collect the protein pellet; and

(e) suspend the protein pellet of the step (d) in a protein pellet solubilization reagent buffer, wherein the reagent buffer is provided with an acid neutralizing agent to neutralize

approximately or greater than 0.25nM acid per micro-gram protein in the pellet to facilitate a desired protein solubilization.

17. The method of Claim 16 further comprises mixing a polysaccharide with the protein pellet of the step (b).

18. The method of protein precipitation according to Claim 16 wherein the protein sample solution contains an ionic detergent SDS.

19. The method of protein precipitation according to Claim 18, wherein the salt agent is in a amount effective to precipitate the detergent present in the protein solution.

20. The method of Claim 16 wherein the salt is provided in a solution with the acid agent.

21. The method according to Claim 16 wherein the pellet solubilization reagent buffer is provided with a pH indicator dye.

22. The method of Claim 16 further comprises vigorously agitating and/or grinding the protein pellet suspended in the pellet solubilization reagent buffer in the step (e).

23. A method of total protein assay, wherein the protein sample contains one or more of non-protein agents selected from a group consisting of an anionic detergent, a cationic detergent, a non-ionic detergent, a zwitterionic detergent, a sulfobutane, a lipid, a natural product, a salt, and a common laboratory agent, comprises the following steps:

(a) treating the protein sample solution with an acidic agent, and one or both agents selected from a group consisting of a salt and a precipitate-forming agent, wherein the treatment of the protein with the precipitate-forming agent follows the treatment of the protein sample solution with the acidic agent;

(b) centrifuge the protein sample solution of the step (a) at least once to form a tight pellet at the bottom of the tube, remove and discard the supernatant and collect a protein pellet;

(c) suspend the protein pellet of the step (b) with one or more alkaline reagents of a protein assay to produce a characteristic protein reaction; and

(d) compare the color density of the protein color reaction with the color density of a protein reaction of known protein concentration.

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